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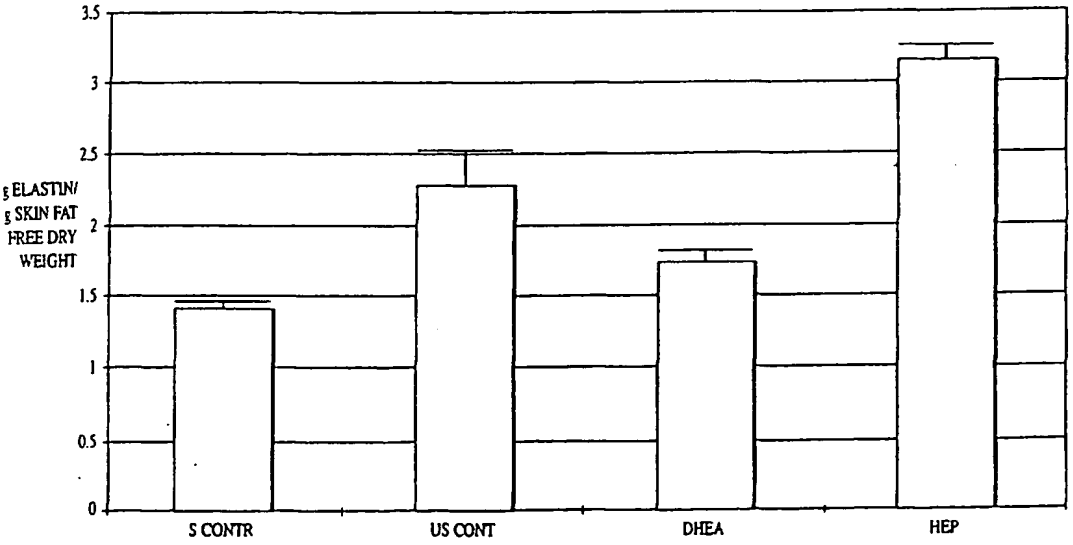
(54) Title: COMPOSITION AND METHOD FOR ENHANCING ELASTICITY OF TISSUE



(57) Abstract: The present invention is directed to a composition and method used to enhance the elasticity and/or appearance of tissue. Specifically, the present invention is directed to a composition formulated from peptides having low molecular weights and which substantially correspond to sequences found in elastin.

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Attempts have been made to use elastin itself as a cosmetic agent, however, the dense cross-linked structure of elastin makes it very difficult to solubilize.

SUMMARY OF THE PRESENT INVENTION

The present invention is directed to compositions that are pharmaceutical, therapeutic, and/or cosmetic in nature. Compositions of the present invention preferably modify or appear to modify the physical characteristics of the tissue to which it is applied.

As described herein, the compound(s) which best accomplish an increase or apparent increase in tissue elasticity and turgor are ones which are analogous to or substantially homologous with portions of elastin. Compounds contemplated within the present invention are those that mimic the action or functionality of amino acid containing peptides or peptide-like compounds of the present invention.

More specifically, the compounds or compositions of the present invention mimic the actions or functionality of a peptide selected from the group consisting of SEQ ID 1, SEQ ID 2, SEQ ID 3, SEQ ID 4, SEQ ID 5 , SEQ ID 6, SEQ ID 7 , SEQ ID 8, SEQ ID 9, SEQ ID 10 , SEQ ID 11, SEQ ID 12, SEQ ID 13, SEQ ID 14, SEQ ID 15, SEQ ID 16, SEQ ID 17, SEQ ID 18, SEQ ID 19, SEQ ID 20, SEQ ID 21, SEQ ID 22, SEQ ID 23, SEQ ID 24, SEQ ID 25, SEQ ID 26, SEQ ID 27, SEQ ID 28, SEQ ID 29, SEQ ID 30, SEQ ID 31, SEQ ID 32, SEQ ID 33, SEQ ID 34, SEQ ID 35, SEQ ID 36, SEQ ID 37, SEQ ID 38, SEQ ID 39, SEQ ID 40, SEQ ID 41, SEQ ID 42, SEQ ID 43, SEQ ID 44, SEQ ID 45, SEQ ID 46, SEQ ID 47, SEQ ID 48, SEQ ID 49, SEQ ID 50, SEQ ID 51, SEQ ID 52, SEQ ID 53, SEQ ID 54, SEQ ID 55, SEQ ID 56, SEQ ID 57, SEQ ID 58, SEQ ID 59, SEQ ID 60, SEQ ID 61, SEQ ID 62, SEQ ID 63, SEQ ID 64, SEQ ID 65, SEQ ID 66, SEQ ID 67, SEQ ID 68, SEQ ID 69, SEQ ID 75, SEQ ID 71, SEQ ID 72, SEQ ID 73, SEQ ID 74 and SEQ ID 75 and their biological equivalents.

Another embodiment of the present invention is directed to a peptide or peptide-like compound having a formula of R_1-Valine-Valine-Proline-R_2, wherein R_1 is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10

appearance of the skin. It is preferable that the administration step be comprised of a number of separate steps which are repeated over a predetermined time (e.g., twice daily for one week). It is preferable that the predetermined time exceeds one week of daily administration of the compound, more preferably two weeks, and most preferably at least a month of daily topical application (with twice daily of the peptide administration over the month being even more preferable).

The compounds and agents described herein are preferably administered at an effective concentration within a therapeutic, pharmaceutical or cosmetic composition. The therapeutically effective concentration of the compound(s) (i.e. the peptide or peptide-like compounds) is preferably in a range of about .0002% to about 90% by weight, more preferably in a range of about .05% to about 50% by weight, even more preferably in a range of about 0.5% to about 10%, even more preferably in the range of about 1.0% to about 2.0%, and even more preferably about 1.8% by weight.

The composition of the present invention can be formulated as a cosmetic preparation to be applied topically to the skin, such as in an emulsion, lotion, spray, powder, ointment, cream, or foam or in other suitable pharmaceutical vehicles or carriers commonly known in the art for other types of administration (e.g., oral or subcutaneous). The delivery system of the present invention is preferably a topical delivery system but also may be a subcutaneous, transcutaneous, oral, nasal, aerosol, or patch. The compositions of the present invention have many other applications. For example, they may also be used to coat surgical devices such as stents and the like.

The composition of the present invention may be suitable to treat a variety of diseases or conditions selected from the group consisting of conditions or diseases of the skin, tendons, sheaths and/or bone, hair, lip, back or spine, brain or nervous system, autoimmune system, lungs, muscle, joints, nails, blood vessels/lymphatics, breast, cartilage, ear, eye, genito-urinary tract, gastrointestinal tract, immunologic systems, ulcerative, blood vessels/heart (e.g., hypertension), and other body systems.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

So that the invention described herein may be more fully understood, the following detailed description is set forth. The description is in no way meant to limit the breadth of the claims, but rather to specifically point out novel aspects of the present invention.

The present invention relates to compositions useful in increasing functionality, elasticity, tone, turgor, and/or appearance of tissue. The present invention is also directed to administering therapeutically effective concentrations of the compositions.

Definitions Useful in Understanding the Invention: As used herein, the term "subject" or "patient " means any mammal in which elastin is utilized for proper tissue function or appearance. The methods herein for use contemplate prophylactic, cosmetic, and curative use.

As used herein, the term "about" means plus or minus 10% of the numerical value of the number with which it is being used. Therefore, about 50% means in the range of 45%-55%. As used herein, the term "Dalton" (or "Da") refers to the unit of mass which is equivalent to the mass of a hydrogen atom (1.66×10^{-24} gram).

Generally speaking, the term "tissue" refers to any aggregation of similarly specialized cells which are united in the performance of a particular function. The term "tissue", as usually used herein, refers to tissue which includes elastin as part of its structure and/or function. For example, connective tissue which is made up of, among other things, collagen fibrils and elastin fibrils satisfies the definition of "tissue". Additionally, since elastin appears to be inherently involved in the visco-elasticity of blood vessels, veins, and arteries, these would be encompassed in the definition of "tissue". The term "skin" is encompassed by the term "tissue" but specifically means the outer integument or covering of the body, including the dermis and the epidermis which rests upon subcutaneous tissue.

hypertension, arteriosclerosis, angina, angiogenesis, myocardial infarction, coronary thrombosis, restenosis post angioplasty, and chronic obstructive pulmonary disease.

The compounds and compositions of the present invention may also be useful as an agent for modifying tissue, especially skin. The term "modify" is used to convey that the present invention changes either the appearance, form, characteristics and/or the physical attributes of the tissue to which it is being provided, applied or administered. The change in form can be reflected in any of the following, alone or in combination: enhanced appearance of the skin; increased softness of the skin; increased turgor of the skin; increased texture of the skin; increased elasticity of the skin; decreased wrinkle formation and increased endogenous elastin production in the skin.

Finally, the term "cosmetic," as used herein, refers to a beautifying substance or preparation which preserves, restores, bestows, simulates, or enhances the appearance of bodily beauty, specifically as it relates to the appearance of tissue or skin.

Initial Methods, Materials, and Formulations: Elastin itself can be used as starting material in the digestion or cleavage methods described herein to arrive at the peptide portion of the composition. This elastin can be derived from a number of sources known in the art. The sequences of the present invention can either be isolated from the digestion pool (and chemically modified if desired) or the peptides may be synthesized with a peptide synthesizer. A particularly useful source of elastin is *ligamentum nuchae. Ligamentum nuchae* contains large amounts of elastin (approximately 70% of the dry weight of this ligament is elastin), especially in proportion to the amount of collagen. Due to the relatively high elastin content and relatively low collagen content, *ligamentum nuchae* is an ideal starting material to use in deriving the elastin peptide fragments of the present invention. *Ligamentum nuchae* may be cleaned first using a procedure similar to that disclosed in U.S. Patent No. 5,028,695, the cleaning portion of which is incorporated herein by reference thereto. Although a preferred source of starting elastin is *ligamentum nuchae*, other ligaments, tendons, connective tissue, tissue, and synthetic sources may also be used. For example, the arteries and lungs, and other animal tissue, especially those which have significant amounts of elastin, can be used (e.g., rat, sheep, and porcine aorta can be used as a

weight of the elastin residue is stable. The elastin residue is then milled in a Willey mill through a 40-mesh screen followed by a 60-mesh screen.

For the thermolysin digestion, three times re-crystallized thermolysin product from CalBiochem (10394 Pacific Center Court, San Diego, CA 92121) was used. The thermolysin preparation contains sufficient calcium to ensure maximal activity of the enzyme. The thermolysin digestion is done as follows: a waterbath is brought to a 55 °C temperature with a rotary shaker and five grams of the finely milled largely insoluble elastin residue is hydrated with one liter of DDW for fifteen minutes at room temperature. After hydration, the one liter of DDW which contains the five grams of elastin is placed in the 55 C bath and the pH of the elastin/water mixture is brought to between 7 and 8 with 10% methylamine. Fifty milligrams of thermolysin (bacillus thermoproteolyticus) is added directly to the elastin/water mixture. The thermolysin contains about 60% protein (60.2%), about 13% (13.2%) sodium acetate, and about 25% (25.3%) calcium acetate, with a specific activity of about 8,720 I.U./mg dry weight. The pH of the elastin water mixture is monitored with a pH meter or pH stat and adjusted with 10% methylamine to keep the pH between 6.8 and 7.5. The digestion is allowed to continue for 75 minutes. Concentrated hydrochloric acid is then added to adjust the pH to 3.0 to terminate the digestion.

After digestion is terminated, the digested product is preferably filtered through a PM 10 Diaflow 10,000 molecular weight cut-off ultra-filtration membrane to filter out any protein or peptides exceeding about 10,000 Da molecular weight. The resulting supernatant is a derived composition comprised of peptides having a molecular weight of less than about 10,000 Da. This step is useful in removing any unwanted higher molecular weight material from the compositions of the present invention. This may be particularly useful in removing any potentially harmful higher molecular weight agents, such as prions or other high molecular weight pathogens.

The elastin peptide fragment/water mixture (inclusive of SEQ IDs 1-41 in Table I shown below) which is obtained upon digestion with thermolysin described above is flash evaporated to dryness, redissolved in a small volume of DDW, and if desired, diluted sufficiently with DDW for lyophilization to dryness. In the alternative, rather than

12.	VGPQ	399	Valine-Glycine-Proline-Glutamine
13.	LGA	259	Leucine-Glycine-Alanine
14.	VGPA	342	Valine-Glycine-Proline-Alanine
15.	VVPG	370	Valine-Valine-Proline-Glycine
16.	AVPG	342	Alanine-Valine-Proline-Glycine
17.	VVPQ	441	Valine-Valine-Proline-Glutamine
18.	VAARPG	569	Valine-Alanine-Alanine-Arginine-Proline-Glycine
19.	LGAGGAG	501	Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine
20.	AIPG	356	Alanine-Isoleucine-Proline-Glycine
21.	LGPGG	399	Leucine-Glycine-Proline-Glycine-Glycine
22.	AAAQA	430	Alanine-Alanine-Alanine-Glutamine-Alanine
23.	VGVHypG	444	Valine-Glycine-Valine-Hydroxyproline-Glycine
24.	VYPGG	491	Valine-Tyrosine-Proline-Glycine-Glycine
25.	IGGVGG	458	Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine
26.	VAPGVG	498	Valine-Alanine-Proline-Glycine-Valine-Glycine
27.	LGVGG	401	Leucine-Glycine-Valine-Glycine-Glycine
28.	VLPG	384	Valine-Leucine-Proline-Glycine
29.	FRAAA	534	Phenylalanine-Arginine-Alanine-Alanine-Alanine
30.	VGGVPG	484	Valine-Glycine-Glycine-Valine-Proline-Glycine
31.	FGPGG	433	Phenylalanine-Glycine-Proline-Glycine-Glycine
32.*	VGVPG	427	Valine-Glycine-Valine-Proline-Glycine
33.	VLPGAG	512	Valine-Leucine-Proline-Glycine-Alanine-Glycine

TABLE II

Fraction #	Approximate Elution time	Approximate % Change Minus Control
1	5.3 min. – 11.8 min	1%
2	11.8 min – 23.0 min	4%
3	23.0 min – 44.1 min	41%
4	44.1 min – 45.8 min	10%
5	45.8 min – 50.0 min	2%
6	Unfractionalized mixture (SEQ IDs 1-41)	52%

Each of the fractions show an increase in mRNA in RFL-6 cells over the control group. From the test, however, it appears that Fraction #3 alone and/or in combination with other fractions (e.g., as seen with Fraction #6) has a preferred composition when increasing elasticity, turgor, and/or appearance of tissue, specifically skin. Fraction 3 includes SEQ IDs 14-31. It should be noted that in light of the ease in obtaining the unfractionalized mixture (as described above) it may be more preferable to use the unfractionalized mixture than to isolate the most active ingredient.

Fraction or Cluster 3 was sub-fractionated into 10 fractions corresponding to the ten major peaks identified on the HPLC (at 215 nm). Table III below illustrates the green fluorescence intensity as a measure of increased mRNA in RFL-6 cells in response to sub-fractionated portions of Fraction No. 3.

The present invention can be formulated in a number of carrier vehicles, for example, in a spray; an aerosol; a water and an oil-type emulsion; an oil and water-type emulsion; a face cream or body cream; a sun lotion or after-sun lotion; or other topical administration vehicle. U.S. Patent No. 4,327,078, which was referenced earlier, is illustrative of the different types of topical administrations which may be employed to administer a soluble elastin-based derivative, and is incorporated herein by reference for this purpose. The method of administering peptides and formulations of the present invention employs any of a number of known administrative routes such as oral, IV, subcutaneous, transcutaneous, and topical administration. A preferred method of the present invention employs a pharmaceutical or cosmetic composition which enhances the physical appearance of and/or the elasticity of tissue. Compositions of the present invention may be in the form of a peptide or peptides in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, bio-compatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones. Pharmaceutically-acceptable carriers may also be comprised of excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of *Remington's Pharmaceutical Sciences*. The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

With the aforementioned wide-spread applicability in mind, a number of peptide or peptide-like compounds were isolated and/or synthesized and analyzed as potential therapeutic, pharmaceutical, or cosmetic agents.

As can be seen from Fig. 1, the topical treatment with a composition which included peptide fragments (i.e., SEQ IDs 1-41) at a concentration of about 1.3% (wt/wt) of

by an increase in vascular response. In Fig. 2, fixed tissue sections of rat skin were labeled with fluorescein conjugated antifibronectin antibodies. Fig. 2a is a representative sample from the unshaven control tissue; Fig. 2b is a representative sample from the shaven control sample; and Fig. 2c is a representative sample of the tissue which received DHEA topical treatment. Finally, Fig. 2d received treatment with the present invention in a topical form in accordance with the samples discussed above with regard to Fig. 1. The dermal layer in the control panels (Figs. 2a and 2b) is relatively uniform and thin compared to the thickness of both Figs. 2c and 2d. For convenience, in each of panels Figs. 2a - 2 d, the dermal layer is bracketed. Surprisingly, panel Fig. 2d illustrates an increased concentration of capillary venules in the subdermal region. The capillary venules are shown in this figure as brightly stained oval bodies that lie beneath the dermal layer. The increase in the concentration of endothelial cells in the subdermal region indicates an increase in capillary density and therefore illustrates the potential for the peptides and formulations of the present invention to be used for the formation of blood vessels or capillary venules. Thus, compositions of the present invention may be useful in neovascularization or angiogenesis.

Modification of Active Peptides: The bar graph of Fig. 3 illustrates the effect of modifying sequences in a variety of ways. The results of modifying SEQ ID 17 (what appears to be the most active peptide for many purposes) provide important information on the impact of such modifications. For instance, modification of SEQ ID NO 17 which result in SEQ ID NOs 42 and 43 appear to adversely impact the suitability for these purposes. SEQ ID 4 (LG) resulted in about an 8% CPM above the control; SEQ ID 17 (VVPQ) resulted in about a 28% CPM above the control; SEQ ID 19 (LGAGGAG) resulted in about an 18% CPM above the control; SEQ ID 42 (VVPQ-NH_2) resulted in about a 1% CPM above the control; SEQ ID 43 (Acetyl-VVPQ) resulted in about a 1% CPM above the control; SEQ ID 48 (GAVVPQ--NH_2) resulted in about a 25% CPM above the control; and SEQ ID 44 (Acetyl-GAVVPQ--NH_2) resulted in about a 5% CPM above the controls. From Fig. 3 and the genetic expression data presented herein, it appears that the synthetic peptide SEQ ID 17 appears to have the same or nearly the same activity as SEQ ID 17 isolated from the HPLC fractionalization. Accordingly, focus should be placed upon this peptide. It would also appear that a GA residue attached to the N-terminus of the SEQ ID 42 (resulting in SEQ ID

TABLE IV (VVPQ derived peptides)

SEQ #	PEPTIDE	MOL WT	NAME (N- to C-terminal)
42	$VVPQNH_2$	448	Alanine-Valine-Proline-Glutamine-Amide
43	$(CH_3CO)VVPQ$	475	Acetyl-Valine-Valine-Proline-Glutamine
44	(CH_3CO) $GAVVPQNH_2$	610	Acetyl-Glycine-Alanine-Valine-Valine-Proline-Glutamine-Amide
45	AVVPQ	512	Alanine-Valine-Valine-Proline-Glutamine
46	GAVVPQ	569	Glycine-Alanine-Valine-Valine-Proline-Glutamine
47	$AVVPQNH_2$	519	Alanine-Valine-Valine-Proline-Glutamine-amide
48	$GAVVPQNH_2$	576	Glycine-Alanine-Valine-Valine-Proline-Glutamine-amide
49	CVVPQC	647	Cysteine-Valine-Valine-Proline-Glutamine-Cysteine
50	CAVVPQC	718	Cysteine-Alanine-Valine-Valine-Proline-Glutamine-Cysteine
51	CGAVVPQC	775	Cysteine-Glycine-Alanine-Valine-Valine-Proline-Glutamine-Cysteine
52	Cu / C V V P Q C	64 647	Copper Cysteine-Valine-Valine-Proline-Glutamine-Cysteine
53	Cu / C A V V P Q C	64 718	Copper Cysteine-Alanine-Valine-Valine-Proline-Glutamine-Cysteine
54	Cu / C G A V V P Q C	64 775	Copper Cysteine-Glycine-Alanine-Valine-Valine-Proline-Glutamine-Cysteine

Based on the information gleaned through testing of derivatives and genetic expression data, "VVP" appears to be an important residue.. SEQ ID 55 was synthesized to

Since SEQ ID 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine) also indicated enhanced activity, it was used as a base model for the synthesis of the peptides shown in Table VI below.

TABLE VI (LGAGGAG derived peptides)

SEQ #	PEPTIDE	MOL WT	NAME (N- to C-terminal)
66	LGAGGAGV	600	Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine
67	LGAGGAGVL	713	Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Leucine
68	LGAGGAGVNH$_2$	607	Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Amide
69	LGAGGAGVLNH$_2$	720	Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Leucine-Amide
70	CLGAGGAGC	707	Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Cysteine
71	CLGAGGAGVC	806	Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Cysteine
72	CLGAGGAGVLC	919	Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Leucine-Cysteine
73	Cu CLGAGGAGC	64 707	Copper Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Cysteine
74	Cu CLGAGGAGVC	64 806	Copper Cysteine- Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Cysteine
75	Cu CLGAGGAGVLC	64 919	Copper Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Leucine-Cysteine

arrays probed with ^{32}P-labeled cDNA obtained from total RNA isolated from VVPQ-treated and untreated skin fibroblasts were analyzed using ImageQuant (Sunnyvale, CA) software.

In the DNA array analysis, the autoradiographic signal obtained from each duplicate cDNA set on each filter is compared to the control cDNAs on the same filter. This intensity ratio is then compared for the same duplicate cDNAs between filters. Increased recovery of signal using a cDNA probe from RNA isolated from treated fibroblasts is interpreted as increased mRNA levels. Decreased mRNA levels are observed as a decrease in signal. There were instances where there was no significant hybridization of labeled cDNA to membrane bound duplicate cDNA spots. All array signals that indicated up or down regulation of steady state mRNA levels of at least two-fold in response to VVPQ treatment were identified.

It appears that VVPQ treatment results in an alteration in steady state levels of relatively few mRNAs, any many of those upregulated in steady state levels were involved in either apoptosis or DNA repair. These include caspase 10 and RFC4. No particular functional class of mRNAs appear to predominate among the mRNAs which appear to be downregulated in response to VVPQ treatment. The hypothesis is that VVPQ has a significant influence on apoptosis in these human fibroblast cultures, but the mechanism of this influence is only now being elucidated.

The peptide LGAGGAG appears to result in an increased steady state level of many different mRNAs. Of these, many of the encoded proteins again appear to be involved in either apoptosis or DNA repair. Although the number of mRNAs altered by treatment with LGAGGAG was greater in our initial studies than with VVPQ, an influence by both peptides on apoptosis and or DNA repair is similar. The larger and distinctly different influence of LGAGGAG, however, appears to be on mRNAs encoding proteins involved in cell to cell communication. A large percentageof mRNAs experinced increased steady state levels in response to treatment of fibroblasts with LGAGGAG. These include mRNAs encoding proteins such as endothelin 2, oncostatin, TGF- and BMP2A. Interestingly, the levels of only a few mRNAs were noted to be significantly down-regulated in skin fibroblasts following treatment with LGAGGAG.

MATERIALS, METHODS, & RESULTS

Elastin mRNA levels. To test the ability of HEP to stimulate the expression of the elastin gene, an rtPCR assay was developed to amplify newly synthesized elastin mRNA. Adult human dermal fibroblasts were grown to approximately 80% confluence prior to being incubated with 10 μg/mL HEP for 2, 4, and 6 hours. A control sample having received no HEP was also run in parallel. After the incubation period, dermal fibroblasts were lysed and total RNA was purified from each sample. Total RNA was quantified and subjected to rtPCR using forward and reverse primers complementary to human elastin mRNA. Amplified cDNA products were then subjected to electrophoresis at 150 volts for 30 min. on a 2% TBE agarose gel containing ethidium bromide. The gel was then photographed and bands were quantified using the Kodak 1D Gel Electrophoresis software. Fig. 4 illustrates this elastin rtPCR assay. Lane (1) shows the net intensity of amplified elastin mRNA from non-treated cells. Lane (2) shows the net intensity of amplified elastin mRNA from treated cells after 2 hours incubation with HEP. Lane (3) shows the net intensity of amplified elastin mRNA from treated cells after 4 hours incubation with HEP. Lane (4) shows the net intensity of amplified elastin mRNA from treated cells after 6 hours incubation with HEP. The net intensities of the PCR products indicate that HEP stimulates nearly a two-fold increase in elastin mRNA expression just after 2 hours of incubating adult human dermal fibroblasts with HEP. Results from this assay indicate that HEP causes an upregulation of the elastin gene in adult human dermal fibroblasts within hours. Specifically, HEP induces nearly a two-fold increase in elastin mRNA after only two hours incubation with adult human dermal fibroblasts as compared to control samples. The stimulus, however, appears to be somewhat transient and elastin mRNA levels begin to decrease in dermal fibroblasts after 4 and 6 hours incubation with HEP.

Tropoelastin levels. Newly synthesized tropoelastin protein levels were measured by quantitative immunocytochemistry in HEP-treated adult human dermal fibroblasts using an LSC (laser scanning cytometer) and a fluorescein-labeled anti-tropoelastin antibody. Adult human dermal fibroblasts were grown to approximately 80% confluence on glass coverslips before being incubated with 10g/mL HEP for 0, 2, 4, 8 and 24 hours. After incubation, 100 μL of 1:100 diluted whole rabbit serum containing anti-human tropoelastin antibodies was added to the dermal fibroblasts after having been fixed in 70%

increased cell density after six weeks of twice daily use of Elastica Skin Cream. Initial quantitative morphometric determinations estimate an enhanced cellularity of approximately 15%. The differences can be seen in H&E and Verhoff stained tissue sections of before and after treatment punch biopsies shown in Fig. 6.

DISCUSSION: Both rtPCR and immunocytochemistry confirm that HEP stimulates production of tropoelastin in adult human dermal fibroblasts in vitro. Quantitative morphometric data obtained from histologies of patient punch biopsies confirm that the HEP cream stimulates an increase in dermal cellularity. It is quite possible that increased tropoelastin levels and dermal cellularity combine to enhance skin viscoelasticity thereby causing a faster recovery rate in treated versus non-treated stretched skin. Together these results offer a plausible explanation for observed increases in skin elasticity through Cutometer measurements. While not wishing to be bound by theory, it appears that the peptides of the present invention increase skin elasticity when applied topically to the skin. Moreover, this increase in elasticity translates to a lessening in the appearance of fine lines and wrinkles as seen in before and after treatment photographs. Furthermore, there were no substantial side effects such as redness, peeling, irritation or sun sensitivity in conjunction with use of the composition(s) of the present invention.

Applications of the Present Invention: The peptide or peptide-like compounds of the present invention, as well as their corresponding therapeutic compositions, are expected to have a variety of important applications. The following descriptions provide a brief summary of the conditions these peptide(s) are likely to benefit.

Skin conditions: There are many skin conditions and diseases which would benefit from elastin treatment. Beyond the obvious cosmetic applications (i.e., increased tone, turgor, and appearance), enhanced elastin production will produce long-term beneficial results. For example, the inherited disease Scleraderma is characterized by a thickening and stiffening of the skin, and cutaneous ulcers due to the overproduction of collagen (there are a number of diseases which involve overproduction of collagen and which seem to have an adverse effect on elastin production/content and compromise the tissue). This disease can also have systemic effects on organs and blood vessels. The stiffness and difficulty in motion along with the cutaneous ulcers would benefit greatly from incorporation of elastin into the

ligament tears and tendon tears will heal faster with supplemental elastin provided by the elastin peptides of the present invention. These tissues may even become stronger as a result of the expected stimulation of elastin production accompanying this treatment. Additionally, cartilage growth abnormalities may be corrected by application of elastin peptides of the present invention.

Treatment with the compound of the present invention will also be useful in veterinary medicine for skin ulcerations in livestock such as horses and cattle. Hoof problems can be very painful and problematic for horses and other hoofed animals. Hoof conditions may benefit from increased keratinocyte production seen with certain compositions of the present invention.

Hair: Hair growth, color, and removal can all be improved by treatment with elastin peptides which will make the hair stronger, more shiny, and improve the condition and healing of irritated skin upon removal of unwanted hair. Premature graying of hair may also be due to decreased elastin.

Lips: Chapped lips and chronic dermatitis or inflammation of the lips can be greatly improved upon treatment with elastin peptides of the present invention. Long-term relief would be a potential benefit from the stimulation of endogenous elastin in these tissues.

Back: The breakdown of elastin in the spine can contribute to herniated disks and lead to acute and/or chronic pain. Replacing elastin with peptides of the present invention along with the stimulation of endogenous elastin could result in improved healing of the disk and reduce or eliminate the pain associated with this condition, especially when combined with other treatments, such as steroids.

Brain and nervous system: In nerve compression syndromes, treatment with elastin peptides of the present invention will likely stimulate endogenous elastin production in certain neurological conditions and promote revascularization after stroke and neural tissue transplants. This revascularization could greatly improve the clinical outcome of these treatments.

Nails: Elastin is useful in treating and preventing nail brittleness, split nails, and to enhance the hardness of nails in general. Nails are comprised of flattened epidermal cells and have a high concentration of elastin in the nail bed. Thus, increasing the elastin content of these cells will result in a stronger and more flexible nail.

Blood vessels/ lymphatics: Elastin is an important constituent of vessels. Therefore, application of the peptides to the affected tissues in vascular diseases would appear to result in beneficial therapy. The vascular diseases contemplated include those which involve abnormalities of arteries or veins including atherosclerotic occlusive disease, chronic venous insufficiency, diabetic vasculitis (inflammation of a vessel caused by diabetes), fibrotic mediastinitis associated with superior vena cava syndrome (an exuberant inflammatory sclerogenic process of infectious, rheumatic, hemorrhagic, or undetermined origin, often accompanied by obstruction of mediastinal structure, especially the vena cava), varicose veins, temporal arteritis, stasis dermatitis, and lymphedema (including elephantiasis, which is a chronic unilateral or bilateral edema of the extremities due to accumulation of interstitial fluid as a result of the stasis of lymph, which is caused by an obstruction of the lymph vessels).

Breast: Capsule contractures secondary to breast implants are disorders of fibers and are conditions of fixed high resistance (rigidity) to passive stretch of a muscle. Fibrocystic disease, selected cases of breast cancer where there is tissue loss may also benefit from treatment with elastin peptides.

Cartilage growth: Transformation of hyaline cartilage to elastin cartilage in remaking of structures such as an ear, nose, larynx or any structure in which elastic cartilage would be beneficial, could be aided by treatment with elastin peptides.

Ear: Chronic serous otitis media and hearing loss secondary to otitis media as well as other diseases causing scarring of the ear drum can benefit from replacement of elastin which can serve to repair scarred ear drum tissue caused by these chronic infections.

cirrhosis, diffuse and interlacing bands of fibrous tissue form and replace the normal liver lobules.

Immunology: Enhancement of the immune response through cytokine activation as well as suppression of immunity for prevention of transplant rejection and for treatment of autoimmune disorders may be mediated by altering elastin levels. It has been shown that human activated lymphocytes express the elastin-laminin receptor. The expression of the elastin-laminin receptor is a general property of most activated human lymphocytes, but is dependent upon lymphocyte subsets. Elastin peptides activate these receptors and trigger the stimulation of biosynthesis and release of an elastase.

Ulcerations: Ulcers are defects or excavations of the surface of an organ or tissue, produced by the sloughing of inflammatory tissue. Common ulcerative disorders include esophageal, duodenal, and gastric ulcers. It is believed that providing ulcerative tissues with elastin will speed the healing of the affected tissue and possibly even strengthen the tissue by stimulating endogenous elastin production.

Blood Vessels/Heart: Since large amounts of elastin are found in the walls of blood vessels, particularly in the arch of the aorta near the heart, it is important to maintain the normal healthy balance of elastin in blood vessels and other vessels (such as lymph vessels). Additionally, in pulmonary tissues, the subendothelium is comprised of the internal elastic lamina, a layer which normally separates the endothelium from the underlying smooth muscle cells. In many cardiac diseases such as congestive heart failure, coronary artery disease, homocystinuria, restrictive pericarditis, sclerosing endocarditis, supra ventricular aortic stenosis, this internal elastic lamina is compromised due to the breakdown of elastin resulting in a remodeling of this matrix layer. This breakdown is often the result of an imbalance in enzyme(s) (such as elastase) which degrade elastin. In some cases, such as in Marfan's syndrome, the elastin malformations are due to an autosomal dominant, congenital disorder affecting connective tissue. Thus, providing affected tissue with normal elastin peptides may be a useful treatment for strengthening the connective tissue in individuals with Marfan's syndrome.

physiologically active amount of the therapeutic mixture may be maintained at the site of the vascular injury, usually for at least one day and up to a week or more.

In Combination with other Skin Enhancing Agents:

In light of the favorable results observed with the elastin peptide composition and the potential applications, the peptide or peptide-like compositions were considered in conjunction (e.g., stepwise administration) or in combination (e.g., a mixture with other skin enhancing agents). Vitamin C (ascorbic acid) and its derivatives are other compounds which have been topically applied as the active ingredient for the treatment of various skin conditions and which would appear useful in combination with the peptides in SEQ IDs 1-75. U.S. Pat. No. 4,983,382 describes the preparation of stabilized ascorbic acid compositions for topical application. It is well known that ascorbic acid (or Vitamin C as it is synonomously referred to herein) is essential to the maintenance of a healthy and attractive skin appearance in humans. Vitamin C helps to stimulate and regulate the production of collagen in human skin tissue thus retarding the formation of wrinkles and otherwise helping to avoid a prematurely aged look to skin. This, in turn, helps to maintain a healthier and younger looking appearance longer. Vitamin C also acts to help prevent or minimize lipid oxidation and other forms of cellular damage resulting from prolonged exposure to the sun's ultraviolet rays, further counteracting premature aging of the skin. It is believed further still that ascorbic acid helps to inhibit (i) the formation of melanin which leads to skin discoloration during the aging process, and (ii) the release of histamine from cellular membranes believed to be responsible for many allergenic reactions, particularly among individuals having so-called sensitive skin. See also, U.S. Pat. Nos. 5,140,043 and 5,122,536, which are incorporated herein by reference.

Another skin enhancing agent that would appear to be suitable in combination or conjunction with peptide SEQ IDs 1-75 would be salicylic acid. It is known to use salicyclic acid for the treatment of acne, see for example, U.S. Pat. Nos. 4,891,227 and 4,891,228, to Thaman et al., the disclosures of which are incorporated herein. Further, salicylic acid has been used for the removal of wart, corns and calluses; for the treatment of psoriasis, seborrheic dermatitis and dandruff; and for the topical treatment of ringworm infection. A listing of commercially available products containing salicylic acid can be found in the Physician's Desk Reference, 45th Edition, 1991, page 323.

300.44. Retin A and RENOVA® are brands of tretinoin (a short for trans-retinoic acid), a substance related to but distinct from vitamin A. RENOVA® differs from Retin A in that it contains a moisturizing cream. Retin A (RENOVA®) produces multiple effects in the skin. In particular, it increases the responsiveness of skin cells to epidermal growth factor (EGF), the natural hormone that stimulates skin growth.

Typical strength of topical tretinoin (Retin A, RENOVA®) creams is 0.025 - 0.1 percent. One study has found that 0.025 percent Retin A may be as effective as 0.05 or 0.1 percent, but with lower incidence of skin irritation. For people with sensitive skin, 0.025% Retin A (RENOVA ®) may be the optimal strength. According to the studies, improvement on tretinoin (Retin A, RENOVA®) may continue for up to a year of continued use.

Early studies by others indicate that RENOVA® or Retin A actually decreases the elasticity of the skin. (See Photodamage by Barbara Gilchrest, published by Blackwell Science (1995)). Contrary to this we have found that in combination with or in conjunction with the peptides or peptide-like compounds of the present invention, an improvement in elasticity is detected when using a tretinoin. A Cutometer (Courage & Khazaka, Germany) is used to quantify skin elasticity. The Cutometer's vacuum probe is placed perpendicular to the skin surface to contact the skin and measure its elasticity. This device then generates data which includes several readings: immediate skin deformation, delayed distention, final deformation, and immediate retraction. The Cutometer SEM 575® (available from Courage & Khazaka, Germany) allows relatively objective determination of elasticity of the skin (See Skin & Allergy News 30(2): 18, 1999). For details on the Cutometer and the testing methods cited herein one is directed to the text Bioengineering of the Skin Methods and Instrumentation (1995 Catalog Number 8374, ISBN: 0849383749, as well as the 1998 version which are both hereby incorporated by reference thereto in their entirety).

A number of volunteers were treated with the hydrolyzed elastin peptides (as described herein, SEQ IDs 1-41) at a 2% weight concentration versus a group of volunteers who used Retin A (actually RENOVA®) first and then applied the 2% hydrolyzed elastin

	R7 (Portion)	+76% *L Cheek	+67% N/A	+68% +81%	+76% +29%	+82% +83%	+73% +70%
Subject 41 (ce) Fitz: III Gender: F-41	R2 (Gross)	+68% *L Cheek	+67% N/A	+73% +26%	+67% +17%*	+60% +9%	+69% +4%
	R5 (Net)	+71% *L Cheek	+77% N/A	+86% +39%*	+89% +50%*	+74% -18%	+68% -14%
	R7 (Portion)	+64% *L Cheek	+64% N/A	+74% +26%*	+72% +39%*	+67% +2%	+58% +2%
Subject: 44 (ph) Fitz: II Gender: F-50	R2 (Gross)	+55% *L Cheek	+65% N/A	+65%	+65%	+42%	+59%
	R5 (Net)	+77% *L Cheek	+79% N/A	+81%	+79%	+76%	+84%
	R7 (Portion)	+65% *L Cheek	+66% N/A	+53%	+66%	+60%	+71%
Subject: 45 (bg) Fitz: III Gender: F-52	R2 (Gross)	+15%	-23%	+2%	8% **+16%	+1%	+3% **+22 % **+5%
	R5 (Net)	+6%	-28%	+25%	+26% **+42%	+38%	+4% **+25 % **-20%
	R7 (Portion)	+6%	-28%	-9%	-16% **+17%	+1%	-22% ***+8 % *-10%

*2nd Biopsy taken L. Cheek
**Monthly interval variances showed a wide range of + and – readings
Please note, this groups' Cutometer readings represent one and three pulls. One pull = total elasticity; three pulls=fatique and recovery

Figure 7 and Table VII illustrate the desirable results obtained when using various embodiments of the present invention. All readings on the Cutometer have been taken in Mode 1, which is constant negative pressure: 5 seconds on, 5 seconds off. The pull of

WHAT IS CLAIMED IS:

1. A composition useful in treating a condition of mammalian tissue, wherein said composition comprises a peptide or biological equivalent thereof, selected from the group consisting of SEQ ID 42, SEQ ID 43, SEQ ID 44, SEQ ID 45, SEQ ID 46, SEQ ID 47, SEQ ID 48, SEQ ID 49, SEQ ID 50, SEQ ID 51, SEQ ID 52, SEQ ID 53, SEQ ID 54, SEQ ID 55, SEQ ID 56, SEQ ID 57, SEQ ID 58, SEQ ID 59 , SEQ ID 60, SEQ ID 61, SEQ ID 62, SEQ ID 63, SEQ ID 64, SEQ ID 65 SEQ ID 66, SEQ ID 67, SEQ ID 68, SEQ ID 69, SEQ ID 70, SEQ ID 71, SEQ ID 72, SEQ ID 73, SEQ ID 74, SEQ ID 75.

2. The composition of claim 1, wherein said composition is a cosmetic preparation.

3. The composition of claim 2, wherein said cosmetic preparation is formulated as a topical preparation to be applied to a patient's skin.

4. The composition of claim 3, wherein said topical preparation is selected from the group consisting of an emulsion, lotion, spray, aerosol, powder, ointment, cream and foam.

5. The composition of claim 1, wherein the mammalian tissue being treated is a blood vessel.

6. The composition of claim 1, wherein the composition is useful for treating a condition selected from the group consisting of hypertension, coronary heart disease, arteriosclerosis, angina, coronary thrombosis, chronic obstructive pulmonary disease, and restenosis post angioplasty.

7. The composition of claim 1, wherein said peptide is useful in improving tissue turgor.

SEQ ID 17, SEQ ID 18, SEQ ID 19, SEQ ID 20, SEQ ID 21, SEQ ID 22, SEQ ID 23, SEQ ID 24, SEQ ID 25, SEQ ID 26, SEQ ID 27, SEQ ID 28, SEQ ID 29, SEQ ID 30, SEQ ID 31, SEQ ID 32, SEQ ID 33, SEQ ID 34, SEQ ID 35, SEQ ID 36, SEQ ID 37, SEQ ID 38, SEQ ID 39, SEQ ID 40, SEQ ID 41, SEQ ID 42, SEQ ID 43, SEQ ID 44, SEQ ID 45, SEQ ID 46, SEQ ID 47, SEQ ID 48, SEQ ID 49, SEQ ID 50, SEQ ID 51, SEQ ID 52, SEQ ID 53, SEQ ID 54, SEQ ID 55, SEQ ID 56, SEQ ID 57, SEQ ID 58, SEQ ID 59, SEQ ID 60, SEQ ID 61, SEQ ID 62, SEQ ID 63, SEQ ID 64, SEQ ID 65, SEQ ID 66, SEQ ID 67, SEQ ID 68, SEQ ID 69, SEQ ID 75, SEQ ID 71, SEQ ID 72, SEQ ID 73, SEQ ID 74, SEQ ID 75, their biological equivalents and combinations of any of SEQ IDs 1-75.

16. The composition of claim 15, wherein said skin enhancing agent is a retinoid.

17. The composition of claim 16, wherein said retinoid is all-trans retinoic acid.

18. The composition of claim 16, wherein said retinoid is Retin A.

19. The composition of claim 16, wherein said retinoid is tretinoin.

20. The composition of claim 15, wherein said peptide is SEQ ID 17.

21. The composition of claim 15, wherein said peptide is SEQ ID 19.

22. The composition of claim 15, wherein said peptide is selected from the group consisting of SEQ ID 1, SEQ ID 2, SEQ ID 3, SEQ ID 4, SEQ ID 5 , SEQ ID 6, SEQ ID 7, SEQ ID 8, SEQ ID 9, SEQ ID 10 , SEQ ID 11, SEQ ID 12, SEQ ID 13, SEQ ID 14, SEQ ID 15, SEQ ID 16, SEQ ID 17, SEQ ID 18, SEQ ID 19, SEQ ID 20, SEQ ID 21, SEQ ID 22, SEQ ID 23, SEQ ID 24, SEQ ID 25, SEQ ID 26, SEQ ID 27, SEQ ID 28, SEQ ID 29, SEQ ID 30, SEQ ID 31, SEQ ID 32, SEQ ID 33, SEQ ID 34, SEQ ID 35, SEQ ID 36, SEQ ID 37, SEQ ID 38, SEQ ID 39, SEQ ID 40, SEQ ID 41.

23. A peptide having a formula of R_1-Valyl-Valyl-Prolyl-R_2, wherein R_1 is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10

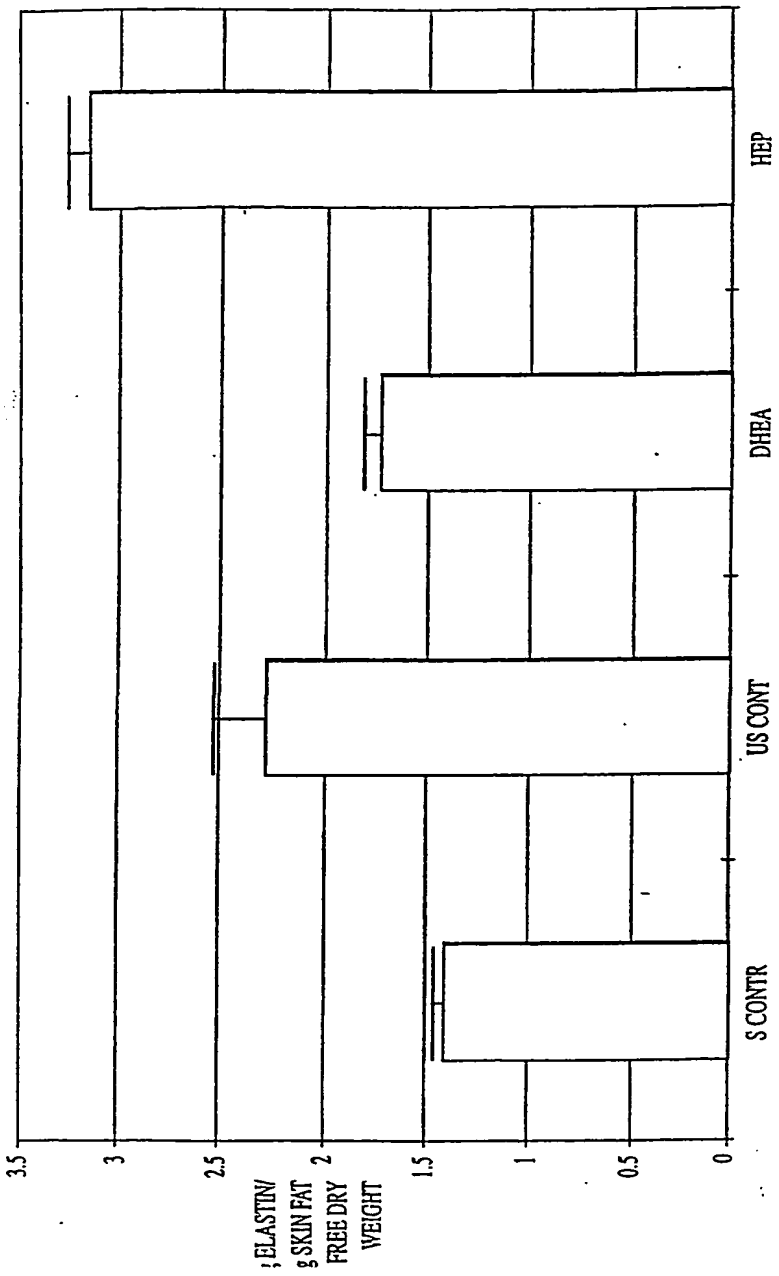
HEP
DHEA
US CONT
S CONTR
3.5
3
2.5
2
1.5
1
0.5
0
ELASTIN/
SKIN FAT
FREE DRY
WEIGHT

FIG. 1

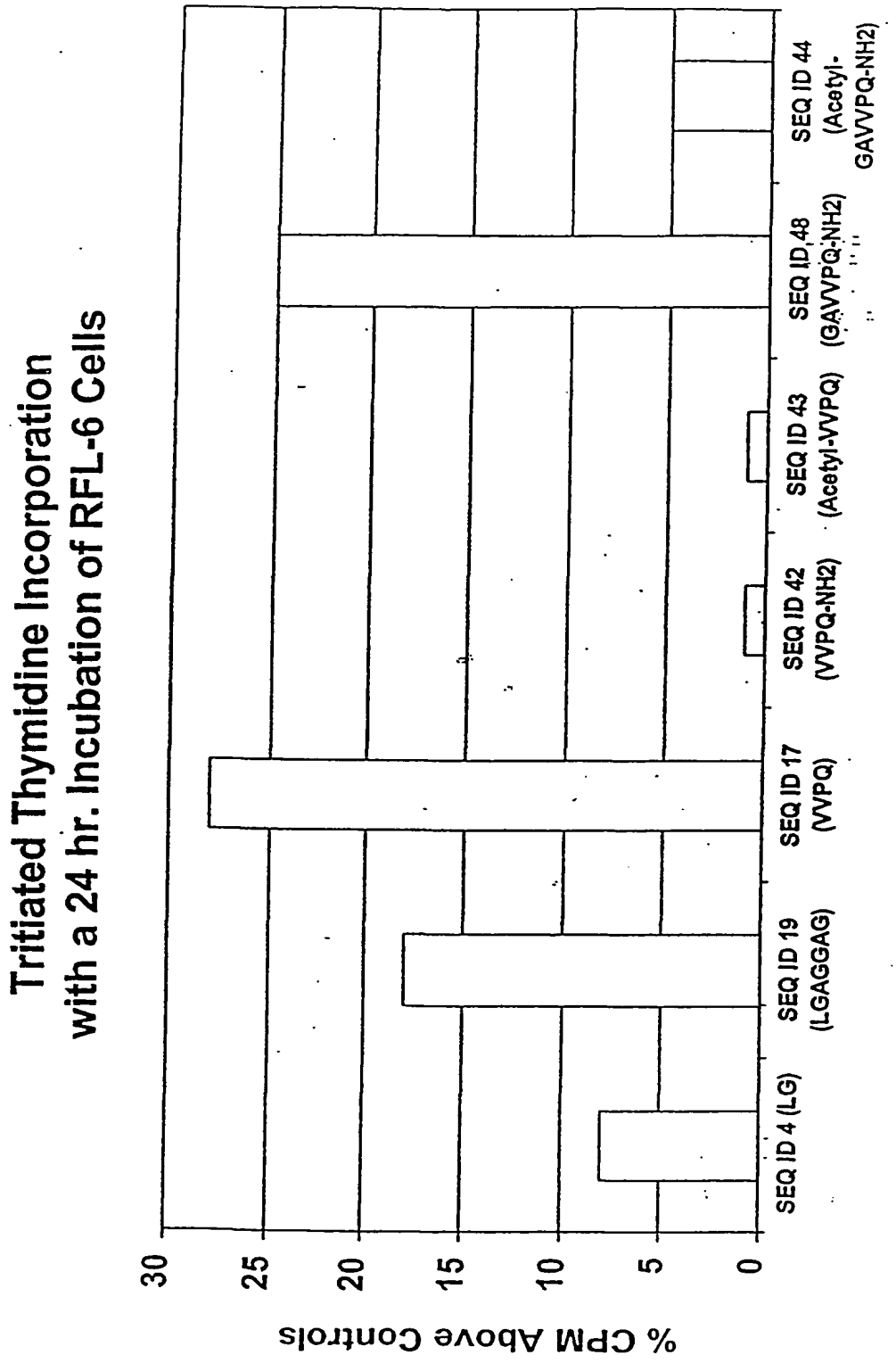
Tritiated Thymidine Incorporation
with a 24 hr. Incubation of RFL-6 Cells
% CPM Above Controls
30
25
20
15
10
5
0
SEQ ID 4 (LG)
SEQ ID 19 (LGAGGAG)
SEQ ID 17 (VVPQ)
SEQ ID 42 (VVPQ-NH2)
SEQ ID 43 (Acetyl-VVPQ)
SEQ ID 48 (GAVVPQ-NH2)
SEQ ID 44 (Acetyl-GAVVPQ-NH2)
Synthetic Peptides (including VVPQ and Analogues of VVPQ)

FIG. 3

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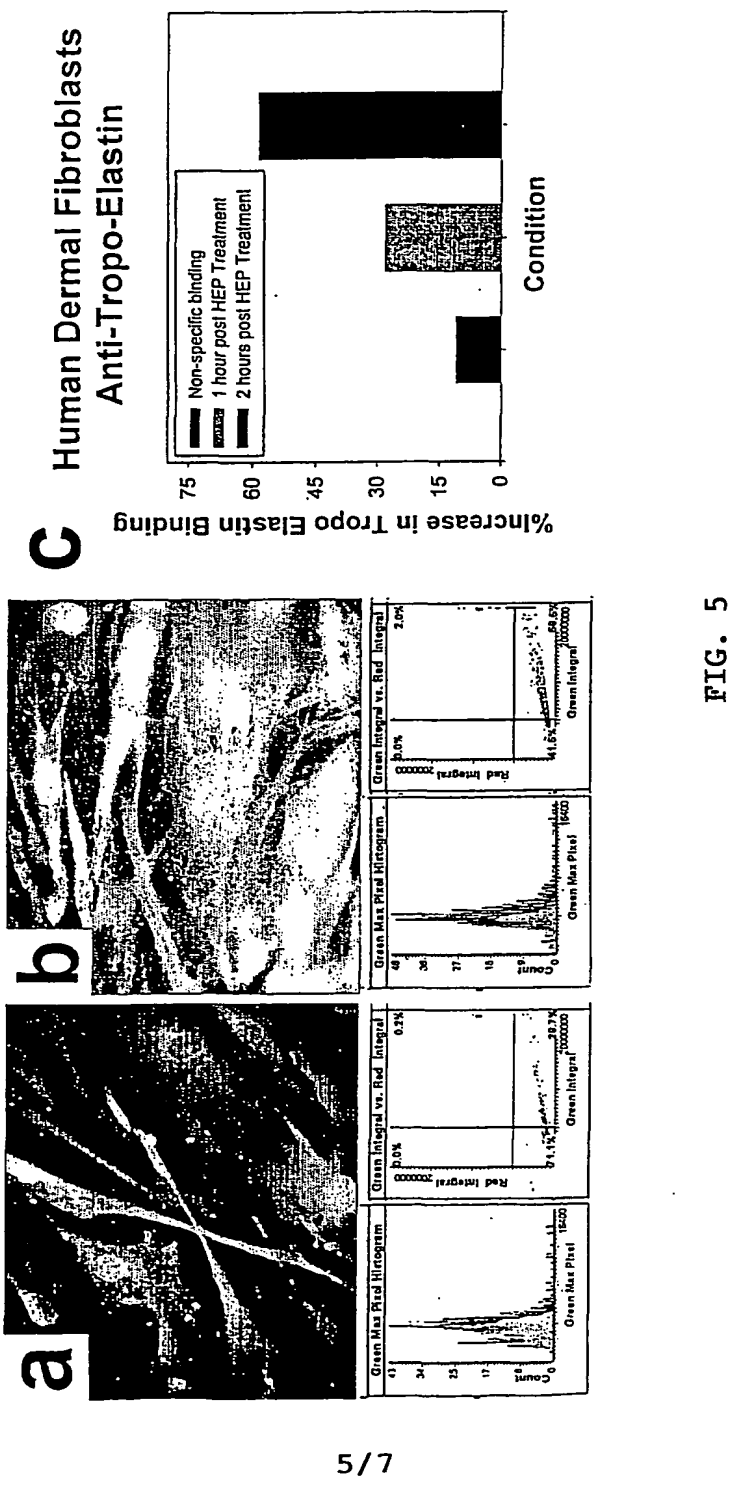

FIG. 5

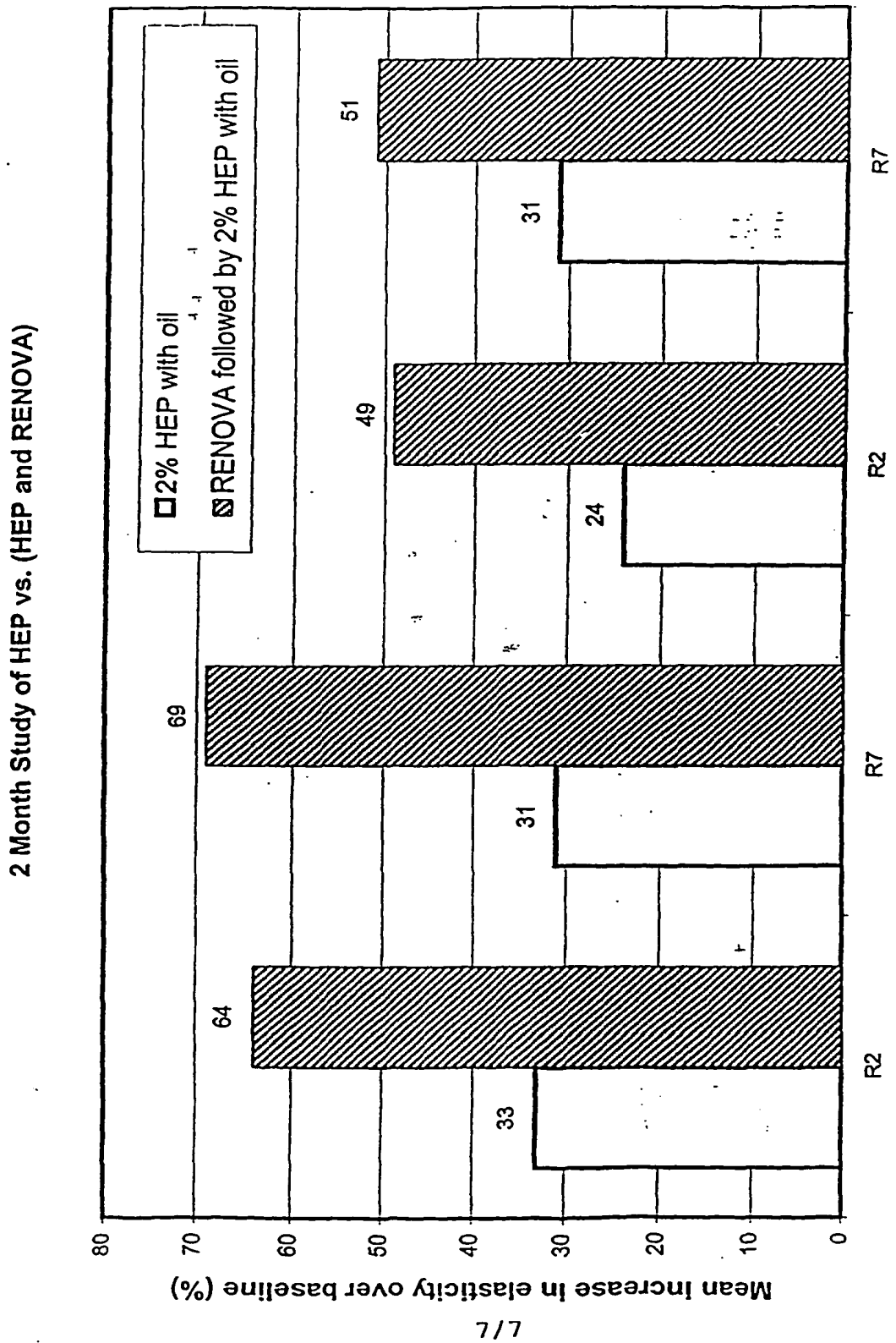
2 Month Study of HEP vs. (HEP and RENOVA)
2% HEP with oil
RENOVA followed by 2% HEP with oil
Mean Increase in elasticity over baseline (%)
0
10
20
30
40
50
60
70
80
64
33
69
31
49
24
51
31
R2
R7
R2
R7
1 Month
2 Month

FIG. 7

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